

IN THE CLAIMS:

Please amend the claims as follows:

1. (Original) An isolated polynucleotide which codes for a polypeptide having the amino acid sequence which is 90 to 100% identical to the amino acid sequences contained in the sequences SEQ ID NO: 2, 3 and 5 or 7, 8 and 10.

2. (Original) The polynucleotide as claimed in claim 1, selected from the group:

a) polynucleotides comprising the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto,

b) polynucleotides comprising nucleotide sequences which correspond to the sequences from a) within the scope of the degeneracy of the genetic code,

c) polynucleotides comprising nucleotide sequences as in a) which comprise functionally neutral sense mutations,

d) polynucleotides which hybridize with the complementary sequences from a) under stringent conditions, where stringent conditions mean washing in 5XSSC at a temperature of from 50 to 65°C,

where the polynucleotides code for a cyanide-tolerant nitrile hydratase.

3. (Original) A polypeptide comprising amino acid sequences which are 90 to 100% identical to the sequences to the sequences SEQ ID NO: 2, 3 and 5 or 7, 8 and 10.

4. (Original) The polypeptide having the activity of cyanide-tolerant nitrile hydratases as claimed in claim 3, whose remaining activity after conversion of methacrylonitrile in the presence of 20 mM (mM=mmol/l) cyanide ions at 20°C after 30 min is at least 90% of the remaining activity of the same enzyme when it has been categorized for the conversion in the absence of cyanide ions under conditions which are otherwise the same.

5. (Original) A probe or primer comprising at least 20 consecutive nucleotides from the sequences SEQ ID NO: 1, 4, 6, 9.

6. (Currently amended) A vector comprising a polynucleotide selected from ~~those claimed in~~

~~claims 1 or 2~~ the group consisting of:

- (a) the polynucleotide of claim 1;
- (b) a polynucleotide comprising the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;
- (c) a polynucleotide comprising nucleotide sequences which correspond to the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, within the scope of the degeneracy of the genetic code, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;
- (d) a polynucleotide comprising the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, which comprise functionally neutral sense mutations, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;
- (e) a polynucleotide which hybridizes with the complementary sequences of nucleotide sequences SEQ ID NO: 1, 4, 6, or 9 under stringent conditions, where stringent conditions mean washing in 5XSSC at a temperature of from 50 to 65°C, where the polynucleotides code for a cyanide-tolerant nitrile hydratase.

7. (Currently amended) A host cell transformed or transfected by the introduction of a polynucleotide ~~as claimed in one or more of claims 1 or 2~~selected from the group consisting of:

- (a) the polynucleotide of claim 1;
- (b) a polynucleotide comprising the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;
- (c) a polynucleotide comprising nucleotide sequences which correspond to the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, within the scope of the degeneracy of the genetic code, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;
- (d) a polynucleotide comprising the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, which comprise functionally neutral sense mutations, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;
- (e) a polynucleotide which hybridizes with the complementary sequences of nucleotide

sequences SEQ ID NO: 1, 4, 6, or 9 under stringent conditions, where stringent conditions mean washing in 5XSSC at a temperature of from 50 to 65°C, where the polynucleotides code for a cyanide-tolerant nitrile hydratase or a vector comprising the polynucleotide.

8. (Original) A host cell transformed by the introduction of a vector as claimed in claim 6.

9. (Currently amended) A process for the enzymatic preparation of amides from nitriles, which comprises the following steps:

a) conversion of a compound comprising nitrile groups using a microbial enzyme (polypeptide) which has nitrile hydratase activity and
b) removal of the amide formed,
employing a cyanide-tolerant nitrile hydratase ~~as claimed in claims 3 or 4~~ selected from the group consisting of:

(1) the polypeptide of claim 3;

(2) a polypeptide having the activity of cyanide-tolerant nitrile hydratases comprising amino acid sequences which are 90 to 100% identical to the sequences to the sequences SEQ ID NO: 2, 3 and 5 or 7, 8 and 10, whose remaining activity after conversion of methacrylonitrile in the presence of 20 mM (mM=mmol/l) cyanide ions at 20°C after 30 min is at least 90% of the remaining activity of the same enzyme when it has been categorized for the conversion in the absence of cyanide ions under conditions which are otherwise the same,
for the conversion of the nitrile to the amide.

10. (Currently amended) The process as claimed in claim 9, ~~characterized in that microorganisms producing and comprising said enzyme as claimed in claims 7 or 8,~~ wherein a host cell transformed or transfected by the introduction of a polynucleotide selected from the group consisting of:

(a) a polynucleotide which codes for a polypeptide having the amino acid sequence which is 90 to 100% identical to the amino acid sequences contained in the sequences SEQ ID NO: 2, 3 and 5 or 7, 8 and 10;

(b) a polynucleotide comprising the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or

nucleotide sequences complementary thereto, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;

(c) a polynucleotide comprising nucleotide sequences which correspond to the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, within the scope of the degeneracy of the genetic code, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;

(d) a polynucleotide comprising the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, which comprise functionally neutral sense mutations, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;

(e) a polynucleotide which hybridizes with the complementary sequences of nucleotide sequences SEQ ID NO: 1, 4, 6, or 9 under stringent conditions, where stringent conditions mean washing in 5XSSC at a temperature of from 50 to 65°C, where the polynucleotides code for a cyanide-tolerant nitrile hydratase
or a vector comprising the polynucleotide, or the lysate thereof, is/are employed.

11. (Currently amended) The process as claimed in claim 10, ~~characterized in that~~ wherein resting cells of the microorganism are employed.

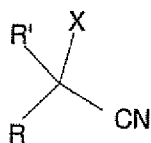
12. (Currently amended) The process as claimed in claim 9, ~~characterized in that~~ wherein a purified nitrile hydratase is employed.

13. (Currently amended) The process as claimed in ~~one or more of claims 9 to 12~~ claim 9, ~~characterized in that~~ wherein the enzyme is derived from microorganisms of the genus *Pseudomonas*.

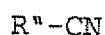
14. (Currently amended) The process as claimed in claim 13, ~~characterized in that~~ wherein the enzyme is derived from employed microorganisms of the species *Pseudomonas putida* or *Pseudomonas marginalis*.

15. (Currently amended) The process as claimed in claim 14, ~~characterized in that~~ wherein the employed microorganisms are deposited under the numbers DSM 16275 and DSM 16276.

16. (Currently amended) The process as claimed in ~~one or more of claims 9 to 15~~ claim 9, characterized in that wherein compounds of the general formula



(I)



(II)

~~In which the meanings are:~~ where

X: is OH, H, alkyl, or NH₂;

R: is H, saturated alkyl radical having 1 to 12 C atoms, branched or unbranched, optionally NH₂-substituted, unsaturated alkyl radicals having a double bond and 1 to 12 C atoms, branched or unbranched, cycloalkyl groups having 3 to 6 C atoms, or alkylene radicals substituted by alkylthio groups, where alkyl here corresponds to a C₁ to C₃ radical, and alkylene corresponds to a divalent C₃ to C₈ radical,

R': is H, or an alkyl having 1 to 3 C atoms,

R'': is a mono- or binuclear unsaturated ring having 6 to 12 C atoms, optionally substituted by one or two alkyl groups (C₁-C₃), Cl, Br, F, or an alkyl nitrile radical having 1 to 6 C atoms,

are converted to the corresponding amides.

17. (Currently amended) The process as claimed in claim 16, ~~characterized in that~~ wherein a compound of the general formula (I) is converted in the presence of hydrocyanic acid or a salt of hydrocyanic acid.

18. (Currently amended) The process as claimed in claim 17, ~~characterized in that~~ wherein the conversion is carried out in the presence of an initial concentration of more than 0.5 mol%

cyanide to 3 mol% cyanide, based on the nitrile employed.

19. (Currently amended) The process as claimed in ~~one or more of claims 9 to 18~~ claim 9, ~~characterized in that~~ wherein 2-amino-4-methylthiobutyronitrile is employed as nitrile.

20. (Currently amended) The process as claimed in ~~one or more of claims 9 to 18~~ claim 9, ~~characterized in that~~ wherein 2-hydroxy-4-methylthiobutyronitrile, where appropriate present in the reaction mixture from the preparation of this nitrile, is employed as nitrile.

21. (Currently amended) The process as claimed in ~~one or more of claims 9 to 18~~ claim 9, ~~characterized in that~~ wherein 2-hydroxy-2-methylpropionitrile is employed as nitrile.

22. (Currently amended) The process as claimed in ~~claims 9 to 21~~ claim 9, ~~characterized in that~~ wherein the amide or the solution comprising the amide is separated from the cells of the biomass, and the amide is hydrolyzed to the corresponding acid.

23. (Currently amended) The process as claimed in ~~claims 9 to 21~~ claim 9, ~~characterized in that~~ wherein the amide or the solution comprising the amide is separated from the cells of the biomass, and the amide is hydrolyzed with alkali metal or alkaline earth metal hydroxides to the salts of the corresponding carboxylic acids.

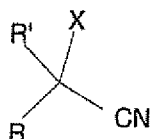
24. (Currently amended) The process as claimed in claim 23, ~~characterized in that~~ wherein MHA amide is hydrolyzed with calcium hydroxide, and the calcium salt is obtained.

25. (Currently amended) The process as claimed in ~~one or more of claims 9 to 24~~ claim 9, where
a) microorganisms of the genus *Pseudomonas* in which isolated polynucleotides which code for polypeptides having the amino acid sequences which are 90 to 100% identical to the amino acid sequences comprised in the sequences with the sequences SEQ ID NO: 2, 3, 5, 7, 8, 10, where the polypeptides have the activity of a cyanide-tolerant nitrile hydratase, enhanced, in particular recombinantly overexpressed, are fermented,

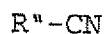
b) the enzyme produced recombinantly and having nitrile hydratase activity is isolated where appropriate from these microorganisms, or a protein fraction comprising this enzyme is prepared, and

c) the microorganisms according to a) or the enzyme or the fraction comprising the latter according to b) is transferred into a medium which comprises a compound comprising nitrile groups of the general formulae (I) and (II).

26. (Currently amended) The process as claimed in ~~one or more of claims 9 to 24~~ claim 10, ~~where host cells as claimed in claims 7 or 8 are employed~~ wherein compounds of the general formula



(I)



(II)

where

X is OH, H, alkyl, or NH₂;

R is H, saturated alkyl radical having 1 to 12 C atoms, branched or unbranched, optionally NH₂-substituted, unsaturated alkyl radicals having a double bond and 1 to 12 C atoms, branched or unbranched, cycloalkyl groups having 3 to 6 C atoms, or alkylene radicals substituted by alkylthio groups, where alkyl here corresponds to a C₁ to C₃ radical, and alkylene corresponds to a divalent C₃ to C₈ radical,

R' is H, or an alkyl having 1 to 3 C atoms,

R'' is a mono- or binuclear unsaturated ring having 6 to 12 C atoms, optionally substituted by one or two alkyl groups (C₁-C₃), Cl, Br, F, or an alkyl nitrile radical having 1 to 6 C atoms,

are converted to the corresponding amides.

27. (Original) A microorganism of the genus *Pseudomonas* deposited under the number DSM 16275 or DSM 16276.

28. (Original) A cyanide-tolerant nitrile hydratase isolated from the strains of the genus *Pseudomonas* deposited under the numbers DSM 16275 and DSM 16276.